REACTOR FOR CULTURING CELLS OR MICROORGANISMS OR FOR DISSOLVING OR SUSPENDING A POWDER IN A LIQUID MEDIUM

BACKGROUND

[0001] The present invention relates to a reactor for culturing cells or microorganisms, or for dissolving or suspending a powder in a liquid medium. In particular, the present invention relates to biotechnology, and specifically, the pharmaceutical industry in which cell cultures and cultures of microorganisms are regularly used as a means of producing therapeutic molecules, and the manufacture of medicaments, the agri-foodstuff and cosmetic fields.

[0002] Cultures of microorganisms such as bacteria, fungi and yeasts are normally produced in bioreactors of large volume allowing mass production on an industrial scale. Plant cell cultures are produced instead in apparatuses of small volume and still remain at the present time at the development stage since the production cost hinders their extension.

[0003] The culturing of animal and human cells constitutes at the present time one of the major thrusts in the pharmaceutical industry, as they allow new therapeutic approaches, such as gene therapy, to be implemented.

[0004] This is because these cell cultures are used either as the actual essence of the medicament within the context of cell therapy or as a means of producing viral vectors used in gene therapy.

[0005] Cell therapy consists in removing certain cell populations from a patient so as to cultivate them and reinject them so as to reestablish or accentuate a particular activity.

[0006] As regards gene therapy, this has the objective of restoring, in the tissues of a patient, a deficient biological function by introducing therapeutic genes by means of appropriate viral vectors.

[0007] At the present time, cell cultures and cultures of microorganisms are produced in reactors having volumes varying between 1 and 5000 liters.

[0008] The reactors known at the present time comprise a glass or stainless steel tank, glass being used more for the small volumes and stainless steel for the larger volumes. They also include a propeller stirrer mounted in the bottom of the tank in order to stir the culture medium and keep the cells in continuous suspension.

[0010] Oxygenation of the culture medium in these known reactors preferably takes place with air, or else with pure oxygen, which is more difficult to regulate and which has the risk of oxidizing the culture medium. Oxygenation may also be carried out with air enriched with 30% oxygen.

[0011] After each culturing of cells or microorganisms, these reactors must be washed, decontaminated and rinsed. They are sterilized before each new culturing, either in an autoclave in the case of small-volume reactors or by injecting steam in the case of larger-volume reactors.

[0012] The operations of washing, maintaining and sterilizing these reactors are steps which are lengthy but essential for their operation.

[0013] In terms of cost, time and human resources, they may represent up to 30% of the operation of the reactor, which is very high.

SUMMARY OF THE INVENTION

[0014] Compared with the aforementioned prior art, aspects of the present invention provide a novel reactor for

culturing cells or microorganisms, which is simple, easy to use and of relatively low manufacturing cost, which is disposable.

[0015] More particularly, the invention provides a disposable reactor that comprises an outer envelope and at least one inner envelope made of plastic, these being placed in one another so as to define, on the one hand, inside the inner envelope, an inner compartment and, on the other hand, between the inner and outer envelopes, at least one outer compartment, the compartments being intended to contain a liquid medium, the envelopes being closed in a sealed manner with respect to the external environment and communicating with one another, which reactor is provided with means for injecting a pressurized gas into the inner compartment and means for removing the gas from the outer compartment in order to stir the liquid medium by making it flow between the compartments.

[0016] The liquid medium is advantageously a culture medium.

[0017] Thus, according to aspects of the present invention, this single-use reactor allows the user to dispense with any washing and maintenance operation, this representing a very substantial saving in terms of time and money.

[0018] According to a preferred embodiment of the present invention, each inner envelope has an opening in its bottom and at least two lateral openings capable of establishing communication between the inner and outer compartments, the opening provided in the bottom of the inner envelope having a much greater cross section than those of the lateral openings.

[0019] The diameter of the lateral openings is preferably

determined so that the openings can allow the culture medium to pass through them at a rate sufficient to break the ascending flux of the medium between the two envelopes, while the medium is being stirred.

[0020] The diameter of the opening at the bottom is determined so that the opening is large enough for the liquid flux to pass mainly through the opening and for the particles of the culture medium to be completely resuspended.

[0021] According to other nonlimiting embodiments of the present invention, there is provide means for injecting gas into the inner compartment. The gas is preferably pure oxygen for oxygenating the culture medium, or nitrogen in order to prevent oxygenation of the medium. Preferably, each inner envelope has a band of perforations extending approximately transversely to the longitudinal direction of the envelope, the perforations favoring transfer of the gas from one compartment to the other. Preferably, the gas injection means comprise a plastic nozzle connected in a sealed manner to the inner envelope so that one of its ends emerges in the inner compartment, the other end preferably emerging outside the reactor. The gas injection and discharge means preferably comprise plastic nozzles connected in a sealed manner to the inner and outer envelopes respectively, so that one of their ends emerges in one of the inner and outer compartments, the other end emerging outside the reactor. Preferably, each gas inlet and outlet is provided with an absolute filter so as to avoid any possible contamination by contaminating agents conveyed by the gas from the liquid medium contained in the envelopes of the reactor.

[0022] Preferably, the outer envelope of the reactor has,

laterally, at least one tap-off for introducing the culture medium into the compartments.

[0023] More preferably, inner and outer envelopes are made of a flexible material, preferably a flexible polyvinyl chloride film, or a polyurethane film.

[0024] Most preferably, the reactor includes a sampling bag made of a flexible plastic material and connected in a sealed manner to the outer envelope so that it communicates with the outer compartment in order that, with the liquid medium being stirred, part of the latter is poured out into the sampling bag.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The description which follows, in conjunction with the appended drawings, given by way of nonlimiting examples, will describe aspects of the present invention and how it may be more clearly understood.

[0026]

[0027] Figure 1 shows a front view of a preferred embodiment of a reactor according to one aspect of the present invention.

[0028] Figure 2 is a table of values of volumes of the reactor of Figure 1.

DETAILED DESCRIPTION

[0029] Figure 1 shows a disposable reactor 100 for culturing cells or microorganisms. Of course, this reactor may be designed to be used for suspending or dissolving solid particles in a liquid medium.

[0030] This reactor 100 comprises an outer envelope 101 and an inner envelope 102 which are made of a flexible plastic and placed in one another so as to define, on the one hand, inside the inner envelope 102, an inner

compartment and, on the other hand, between the inner envelope 102 and outer envelope 101, an outer compartment.

[0031] The inner envelope 102 and outer envelope 101 constitute flexible bags, one inserted inside the other.

[0032] The reactor 100 as shown has a useful volume of about 20 liters, which represents an outer envelope 101 with a width of 360 mm and an inner envelope 102 with a width of about 260 mm.

[0033] The two inner and outer envelopes 101, 102 are positioned with respect to each other so as to be concentric with respect to a longitudinal axis X.

[0034] Of course, reactors of the same type could be provided that have a larger volume, ranging up to at least 400 - 500 liters.

[0035] The envelopes 101, 102 are preferably made of a flexible polyvinyl chloride film, which is high-frequency weldable, inexpensive and has good mechanical strength.

[0036] Provision may also be made for the outer and inner envelopes to be made of polyurethane, which resists heat well and has great mechanical strength.

[0037] The inner envelope 102 and outer envelope 101 are closed in a sealed manner with respect to the external environment and communicate with each other.

[0038] The envelopes 101, 102 are sealed along their upper edges 101a, 102a preferably by high-frequency welding so that the weld joins them together.

[0039] The inner envelope 102 has an opening 104 in its bottom and at least two lateral openings 105 capable of establishing communication between the inner and outer compartments. The opening 104 provided in the bottom of the inner envelope has a much greater cross section than that of the lateral openings.

[0040] More particularly, the opening 104 provided in the bottom of the inner envelope 102 has here a width when flat of 60 mm. This width has been carefully determined so that the opening 104 is large enough for the flux of liquid culture medium to pass mainly through this opening and for the particles, which have settled in the culture medium, to be completely resuspended.

[0041] The diameter of the lateral openings 105 of the inner envelope 102 has been determined so that these lateral openings can allow a sufficient flow of liquid to pass through them so as to break the ascending flux of liquid medium between the two envelopes and prevent the flexible inner envelope 102 from being bent during the ascent of the liquid medium in the inner compartment, thereby making it possible to obtain good homogenization of the culture medium.

[0042] The liquid medium in the reactor 100 is stirred with the aid of means for injecting a pressurized gas into the inner compartment and with the aid of means for discharging the gas from the outer compartment so as to make the culture medium flow between the compartments via the openings 104, 105.

[0043] The term "pressurized gas" is understood here to mean a gas under a slight overpressure with respect to atmospheric pressure (an overpressure of a few mbar is sufficient).

[0044] According to the example shown, the gas injection and discharge means comprise nozzles 103, 103' connected in a sealed manner to the inner envelope 102 and outer envelope 101, respectively, so that one of their ends emerges in one of the inner and outer compartments and the other end emerges outside the reactor.

[0045] The nozzles 103 are intended to be connected to a pressurized gas feed (not shown), preferably a pressurized air feed.

[0046] Thus, the stirring of the medium in the reactor according to the invention is based on fluid mechanics so as to make the culture medium perfectly homogeneous. The pressure exerted in the inner compartment of the reactor causes the culture medium to rise in the outer compartment defined between the two envelopes, the supernatant decanted matter therefore being immediately resuspended.

[0047] When the pressure is released, the level of the liquid medium returns to its initial state in the inner compartment, thereby also causing the medium to be stirred.

[0048] The leaktight connection between the plastic nozzles 103, 103' and the envelopes 102, 101 is made when the two envelopes are welded together.

[0049] The reactor 100 also includes means for injecting a gas, here pure oxygen, into the inner compartment in order to oxygenate the culture medium.

[0050] These pure oxygen injection means may comprise a plastic nozzle, independent of the nozzles 103, 103', which is connected in a sealed manner to the inner envelope of the reactor so that one of its ends emerges in the inner compartment, the other end emerging outside the reactor, in order for it to be connected to a pure oxygen feed.

[0051] According to the embodiment shown, the pressurized gas is injected into the inner compartment simultaneously with the injection of pure oxygen via the same nozzles 103.

[0052] Advantageously, the inner envelope 102 of the reactor includes a band of perforations 106 extending approximately transversely to the longitudinal direction X of the envelope 102. The perforations 106 favor the

transfer of pure oxygen from one compartment to the other.

[0053] The density of the perforations is determined according to the intended degree of oxygenation of the culture medium contained in envelopes. Furthermore, the thermoplastic used to produce the inner and outer envelopes of the reactor is permeable to the gas, and especially to oxygen, so as to increase the area for exchange between the culture medium and the environment and therefore to optimize the oxygenation of the medium.

[0054] The outer envelope 101 includes, laterally, a tap-off, here a sealed plug 107 allowing the culture medium to be introduced and extracted. This tap-off 107 is protected by a suitable adhesive tamper-evident tab and can be opened by perforating the outer envelope in a perfectly sterile manner.

[0055] As figure 1 shows, the reactor furthermore includes at least one plastic pipe 108, which is connected in a sealed manner to the outer envelope (in the same way as the other nozzles) and emerges at one end in the bottom of the inner compartment and at another end outside the reactor, in order to introduce various measurement probes.

[0056] In particular, it is possible to introduce a pH probe or an oxygen probe to check whether sufficient oxygen has been transferred during culturing of the medium contained in the compartments of the reactor. This pipe is made of a thermoplastic and is welded to the inner and outer envelopes in a perfectly sealed manner, preferably by high-frequency welding.

[0057] The temperature in the rector 100 is advantageously regulated with the aid of a vortex tube, which is connected in a sealed manner to the outer envelope and emerges at one end in the bottom of the outer compartment and at the other

end outside the reactor. The vortex tube converts an ordinary compressed-air supply into two streams of air: one hot and the other cold, at a pressure slightly above atmospheric pressure. A throttling valve on the hot outlet of the tube (not shown) makes it possible to adjust the flow rates and temperatures over a continuous range. This heating or cooling system is regulated by a temperature probe (not shown) slipped into the pipe 108.

[0058] Advantageously, the reactor 100 may include a sampling bag (not shown) made of a flexible thermoplastic and connected in a sealed manner by a weld to the outer envelope so that it communicates with the outer compartment. In this way, when the liquid medium is stirred, part of the latter is poured out into the sampling bag. Samples may be taken at any moment, during the culturing of the medium, with the aid of a clip that heat-seals and cuts off a sample bag from the sampling bag in order to recover a defined amount of sample of the liquid medium. This allows sequenced sampling to be carried out.

[0059] However, provision may be made for this reactor not to have such a sampling bag and for the medium to be recovered after culturing by opening the envelopes.

[0060] The reactor is drained with the aid of a pump (not shown) provided on the upstream side with a filter which may be connected to the nozzle 103' shown in Figure 1. This pump is associated with a hollow dip tube (not shown), which makes it possible to sample the medium via the bottom of the reactor 100.

[0061] According to the example shown, the minimum height of the inner envelope 102 has been set at 260 mm and the maximum height of the liquid medium in the envelopes is set here at 900 mm.

[0062] The difference between the widths of the two envelopes 101, 102 was determined by trials so that the inner envelope is wide enough and allows a large area for exchange between the liquid medium and the air or oxygen.

[0063] The volume of the outer compartment, that is to say the space provided between the two envelopes, must be large enough to allow the liquid medium to be properly removed from the inner envelope and for the culture medium to be made perfectly homogeneous.

[0064] The range of volumes for this type of reactor 100 is between 11 and 20 liters, knowing that there is complete homogenization of the medium contained in the reactor, even a 20 liter reactor. The various volumes appear in table 1, shown in Figure 2.

[0065] The reactor 100 is advantageously delivered ready for use, is perfectly sterile and is optionally fitted with all the probes necessary for using it. It may be prefilled with the culture medium.

[0066] Furthermore, a rigid retaining tank may be provided, in which the outer and inner flexible envelopes are suspended with the aid of standard suspension means. This retaining tank is then perfectly sealed and ensures that the system is secure should the outer envelope be inopportunely pierced.

[0067] The present invention is in no way limited to the embodiment described and shown, rather a person skilled in the art will be able to make any variant thereof in accordance with its spirit.

[0068] In particular, provision may be made for the inner and outer envelopes to be made of a rigid plastic.

[0069] The reactor according to the invention may also be provided so as to comprise a number of envelopes greater

than 2, these being imbricated in one another so as to define an inner compartment at the center of the reactor and a plurality of concentric outer compartments surrounding the inner compartment, all of the compartments communicating with one another in order for the culture medium to flow between the compartments.